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RESEARCH PAPER

Poloxamer 407 as a Thermogelling and Adhesive Polymer for Rectal Administration of Short-Chain Fatty Acids

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ABSTRACT

Objectives: *The purpose of the study was to gel a rectal solution of short-chain fatty acids to decrease the loss of active materials in the colonic lumen and thereby optimize their absorption.* Methods: *Five thermogels were prepared with poloxamer 407 at concentrations ranging from 17% to 20%. Their viscosities were measured at room temperature and 37°C, and their gelling temperatures were determined. The adhesive properties of each gel were assessed in vitro at 37°C. Short-chain fatty acid release was studied using Guyot cells.* Results: *From the threshold concentration of 17.5%, the solutions, Newtonian at room temperature (50–80 mPa · s), gelled at 37°C. The higher the concentration, the higher the viscosity (1750 to 49,000 mPa · s), the lower the gelling temperature (27.6°C to 23.4°C), and the stronger the work of adhesion (2.2 to 4.5 mJ). Short-chain fatty acid release from the 18% polymer gel was decreased by 60% compared to the rectal solution.* Conclu-

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sion: *The 18% poloxamer 407 concentration provided a solution that was liquid at room temperature, that gelled at 37°C, possessed adhesive properties, and controlled short-chain fatty acid release.*
KEY WORDS: *Adhesion; Drug release; Poloxamer 407; Short-chain fatty acids; Thermogels for enema.*

INTRODUCTION

The short-chain fatty acids are saturated, alkyl, monocarboxylic acids of 2–6 carbon atoms. They are produced in the colon. They are mainly by-products of anaerobic fermentation of dietary fibers and other saccharides that escape absorption in the small bowel and reach the large bowel. Sodium acetate, propionate, and butyrate account for approximately 85% of short-chain fatty acids formed and are produced in a nearly constant molar ratio of 65/20/15. In the colonic lumen, short-chain fatty acids are the predominant anions (70%) found in high concentration, from about 100 to 200 mmol/L.

Among their various properties, current research has identified the metabolic and intestinal effects of short-chain fatty acids in experimental models and humans (1,2). Hence, physiological rationale justifies the administration of short-chain fatty acids in selected patients with intestinal dysfunctions such as distal ulcerative colitis (3) and short-bowel syndrome (4). Radionuclide studies have clearly demonstrated that enemas can deliver drugs up to the splenic flexure (5,6). The local application of the drug has several advantages over oral therapy, including delivery of high concentrations to the mucosa and better response rates.

Accordingly, we previously elaborated a rectal solution (sterile, neutral, and isoosmolar) containing the three main short-chain fatty acids to reproduce the ionic content of the colonic lumen (7). However, a major disadvantage is the inconvenience of handling an enema or the incapability of retaining it due to disease. Indeed, the liquid form requires bed rest after introduction twice a day (8). These problems may be overcome in part by the use of mucoadhesive gel preparations, which could result in longer persistence in the colon and less interference with daily life activities.

The purpose of the present work was to gel the rectal solution and to make it bioadhesive to decrease the loss of short-chain fatty acids in the colonic lumen, thereby optimizing their absorption, reducing the number of administrations per day, and improving the patient comfort. Gelling of the rectal solution should remain compatible with easy administration of the preparation, which should be fluid at room temperature.

Because of its physicochemical properties, Poloxamer 407 was chosen to gel the short-chain fatty acid solution. Poloxamers are poly(ethylene oxide)–(polypropylene oxide)–poly(ethylene oxide) triblock copolymers that form micelles at low concentrations and clear, thermoreversible gels at high concentrations (9–11). Poloxamer 407 has a nominal molecular weight of 12,220 and a poly(ethylene oxide)/(polypropylene-oxide) ratio of 2:1 by weight. Poloxamer 407 is chemically inert, and one of its main interests results from its very low toxicity (12,13). Poloxamer 407 gels have been used as drug delivery systems for topical, transdermal, ophthalmic, and implantable applications (14–18). The gel formation process is characterized by a sol-gel transition temperature: Below this temperature, the sample is fluid, allowing comfortable and precise delivery; above this temperature, the solution gels.

2. MATERIALS AND METHODS

Preparation of the Formulations

Short-Chain Fatty Acid Solution

Composition of the rectal short-chain fatty acid solution is shown in Table 1. Sodium acetate was purchased from Sigma (Saint-Quentin Fallavier, France); sodium

Table 1

Composition of the Rectal Short-Chain Fatty Acid Solution

	g	mmol
Sodium acetate	4.100	50
Sodium propionate	1.920	20
Sodium butyrate	2.200	20
Sodium chloride	0.585	10
Potassium chloride	2.235	30
Sodium bicarbonate	1.680	20
Acetic acid solution (1%)	To pH 7.00	
Distilled water	To 1000 g	

butyrate and acetic acid were purchased from Merck (Nogent-sur-Marne, France); sodium propionate, sodium chloride, potassium chloride, and sodium bicarbonate were purchased from Cooperation Pharmaceutique Française (Melun, France).

Dissolution of each component was carried out under magnetic stirring. The pH was adjusted to 7.00 with the 1% acetic acid solution.

Poloxamer 407 Gels

Poloxamer 407 (Lutrol® F127) was purchased from BASF (Ludwigshafen, Germany). Five gels were prepared by dissolving 17%, 17.5%, 18%, 19%, and 20% w/w polymer in the short-chain fatty acid solution using the cold method (12). Poloxamer 407 was slowly added under gentle stirring to the short-chain fatty acid solution maintained at 5°C in an ice bath. Poloxamer 407 dissolution required 2 h. As a preservative, a combination of methyl, ethyl, propyl, and butylparahydroxybenzoate plus phenoxyethanol (Phenonip®, SIPCA, Paris, France) was added under stirring at the end of the preparation at a concentration of 0.15%. This classical mixture is known for its broad-spectrum activity, which is effective against gram-negative and gram-positive bacteria, yeasts, and molds over the range pH 3–8. The final pH of the gels was 8.2.

Viscosity Study

A controlled shear rate viscosimeter (Brookfield Rheoset RV type, Labomat Essor, Saint-Denis, France) was used to assess the behavior of each gel. Shear stress (mPa) was recorded as a function of the shear rate (s^{-1}) at room temperature and 37°C on 17-ml samples. The apparent viscosity of each preparation was determined at the shear rate of 3.8 s^{-1} to allow comparison among gels.

Gelling Temperature Determination

Rheological studies were performed with a thermostatically controlled shear stress rheometer (Carri-Med CSL 100, Carri-Med, Ltd., Vincent Lane, England). The cone/plate geometry was used. The cone had a 4 cm diameter and an angle of 3°58′. The shear stress was controlled to maintain a shear rate of 10 s^{-1} shear rate. This value was chosen, regarding the poloxamer 407 thermogel property, to allow precise determination of the gelling temperature. The temperature was increased in steps of 1°C per minute, from 20°C to 40°C to locate the sol/gel transition point.

The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity (Pa · s) as a function of the temperature (°C). Each preparation was tested twice to control the repeatability of the measurement.

Adhesion Study

Adhesion was assessed according to Gurny's method (19). A dynamometer (Lhomargy DY20B, Ivry, France) was used to measure the detachment force between two aluminum/inox pieces of a chamber containing the gels to be tested. The tensile force (daN) was recorded as a function of extension (mm) at an extension rate of 3 mm/min. The results were expressed as work of adhesion (mJ), or work required for breaking the gel/support system, and were calculated by measuring the area under the curve with a digital planimeter (Ottplan 700/710, Kempten, Germany). Each preparation was tested 10 times at 37°C.

Release Study

The short-chain fatty acid release was studied in vitro using Guyot cells (20) (2.5 cm diameter, $n = 6$ per assay). The donor phase (2 g) was either poloxamer 407 gels or the short-chain fatty acid rectal solution used as a control. Assays were carried out at 37°C for 1 h to determine the immediate release of short-chain fatty acids. The membrane between the donor phase and the receptor phase was regenerated cellulose (ref. 18406-047, 0.45-µm porosity, Sartorius, Palaiseau, France).

The receptor phase was stirred distilled water, which was chosen in preference to buffer solutions since the rectal fluid does not exhibit any buffer capacity. The volume (50 ml) was adapted to short-chain fatty acid solubilities in water, which are as follows: sodium acetate 1.25 g/ml, sodium propionate 1 g/ml, and sodium butyrate 0.1 g/ml (at 25°C). Samples of 2 ml were taken in the receptor phase at 10, 15, 30, 45, and 60 min, and short-chain fatty acid concentrations were measured by high-performance liquid chromatography. The mobile phase was composed of distilled water (95%) and methanol (5%) adjusted to pH 2 with perchloric acid. A C18 nucleosyl column (5 µm, 150 mm, Chrompack, Les Ulis, France) was used. The column flow rate was maintained at 0.7 ml/min. The volume injected was 50 µl. The wavelength was set at 210 nm.

Statistics

Data were expressed as mean plus or minus standard error of the mean (SEM). They were analyzed using the *t* test. Differences were considered significant at $P < .05$.

RESULTS

Viscosity of the Gels at a Constant Temperature

Viscosity at Room Temperature

Poloxamer 407 solutions were fluid at room temperature. Every gel had a constant viscosity (Newtonian behavior) that ranged from 50 to 80 mPa · s (at 3.8 s^{-1} shear rate) for 17% to 20% polymer.

Viscosity at 37°C

At 37°C, the behavior of poloxamer 407 solutions changed, depending on the polymer concentration. The 17% solution remained fluid and showed a constant viscosity of 70 mPa · s. From 17.5% concentration, the preparations gelled and showed a shear-thinning (pseudoplastic) behavior. Their apparent viscosity increased with the polymer concentration: It was 1750, 32,500, 39,800, and 49,000 mPa · s (at 3.8 s^{-1} shear rate) for 17.5%, 18%, 19%, and 20% polymer gel, respectively. As an example, the rheogram of the 18% poloxamer 407 gel at 37°C is presented in Fig. 1.

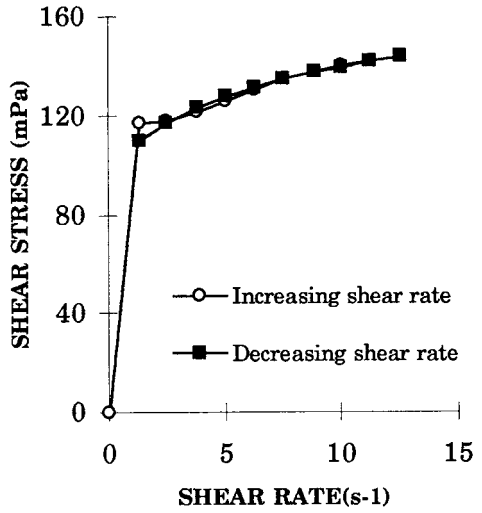

Figure 1. Rheogram of the short-chain fatty acid gel with 18% poloxamer 407 at +37°C.

Gelling Temperature Determination

Figure 2 represents the viscosity (Pa · s) of each gel, measured at a shear rate of 10 s^{-1}, as a function of the temperature (°C). The 17% poloxamer 407 solution did not gel between 20°C and 40°C. From 17.5% polymer, the curves were composed of three phases: In the first part, the viscosity was nearly constant; in the second part, it increased dramatically and reached its maximum; in the third part, it remained constant at the plateau. The gelling temperature, determined graphically as the inflection point of the second part of the curve, decreased as the polymer concentration increased. At the plateau, the maximum viscosity increased as the polymer concentration increased. Values are summarized in Table 2.

Adhesion Measurement

The assessment of the work of adhesion at 37°C showed that the poloxamer preparations possessed adhesive properties that increased with the polymer concentration (Fig. 3). The work of adhesion was very low with 17% polymer and significantly increased from the 17.5% concentration. The work of adhesion was comparable for the 18% and 19% preparations and significantly increased with 20% polymer.

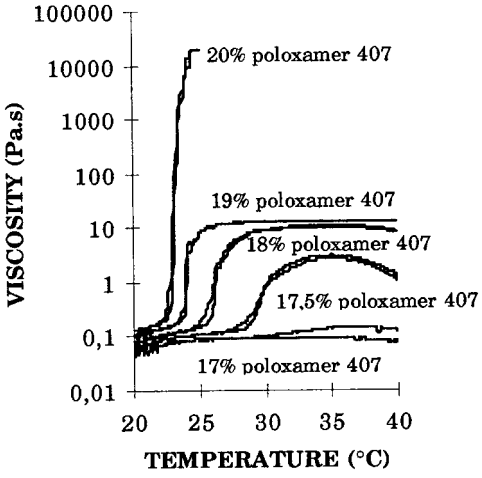

Figure 2. Effect of temperature on the viscosity of short-chain fatty acid gels with 17% to 20% poloxamer 407 measured at 10 s^{-1} shear rate.

Table 2

Effect of the Poloxamer 407 Concentration on the Gelling Temperature and the Maximum Viscosity of the Gels

Poloxamer 407 Concentration (%)	Gelling Temperature (°C)	Maximum Viscosity[a] (Pa · s)
17	—[b]	0.1
17.5	27.6	5
18	26.4	10
19	24.0	30
20	23.4	>10,000

[a] The viscosity was measured at 10 s^{-1} shear rate.
[b] The 17% preparation did not gel between 20°C and 40°C.

In Vitro Release of Short-Chain Fatty Acids

Figure 4 represents the release of sodium butyrate from the rectal solution and the 18% poloxamer 407 gel at 37°C. Compared to the rectal solution, the gel significantly reduced the sodium butyrate diffusion. At 60 min, the area under the curve was 164 ± 3 for the gel versus 414 ± 24 for the solution ($P < .001$).

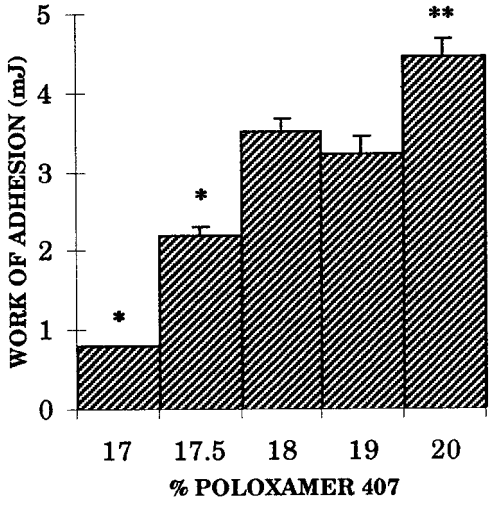

Figure 3. Influence of poloxamer 407 concentration on the work of adhesion measured in vitro at +37°C, mean ± SEM; *$P < .05$ versus every other concentration; **$P < .05$ versus 18% and 19% concentrations.

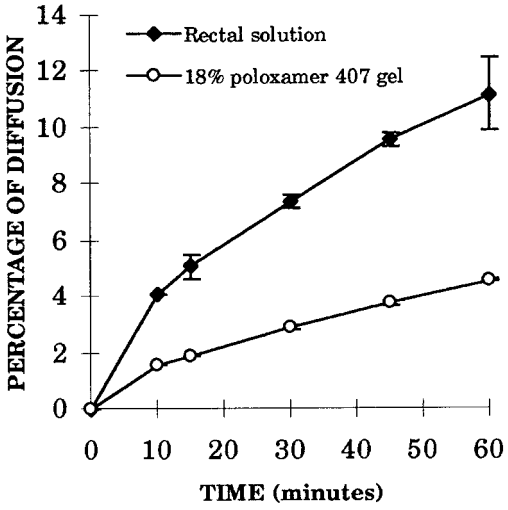

Figure 4. Percentage of diffusion of sodium butyrate from the rectal solution and from the 18% poloxamer 407 gel at +37°C in Guyot cells, mean ± SEM.

DISCUSSION

The sol-gel transition temperature of poloxamer 407 solutions is dependent on the polymer concentration and the ionic content of the solution (12). It can be changed using additives (21), and some inorganic salts, like sodium chloride, lower the sol-gel transition temperature and increase the viscosity of the gels (11). Due to the ionic content of the solution (300 mosm/L), the first objective of our work was to assess whether poloxamer 407 was compatible with its components and to determine the range of concentrations that could provide solutions that were liquid at room temperature and gelled at the physiological temperature. In a preliminary study, concentrations ranging from 11% to 18% w/w were screened and showed that, up to 17%, the solutions of short-chain fatty acids and poloxamer 407 did not gel at physiological temperature (data not shown). Since the 18% solution did gel, concentrations ranging from 17% to 20% were tested to determine precisely from which concentration the preparations could gel at 37°C. The threshold concentration was 17.5%, and the viscosity of the gels increased with the poloxamer 407 concentration.

These data are in agreement with a recent study on 14% to 25% w/w poloxamer 407 solutions in ultrapure water, which showed that below 18%, solutions did not gel, and 18%, 20%, and 22% preparations, newtonian at

20°C, were pseudoplastic at 35°C, with viscosity that increased with the polymer concentration (22). Our determination of the sol-gel transition temperature demonstrated that, the higher the concentration, the lower the transition temperature and the higher the final viscosity. In the same way, an increasing polymer concentration gave an increasing gel strength and a decreasing sol-gel transition temperature in ultrapure water (22).

From the viscosity studies, it was concluded that the lower threshold concentration of poloxamer 407, which provided fluid short-chain fatty acid preparations that gelled at 37°C, was 17.5%. However, viscosity of the 17.5% poloxamer 407 gel at 37°C was considered too low to exhibit a real interest compared to the short-chain fatty acid solution. Furthermore, the sol-gel transition temperatures of the 19% and 20% poloxamer 407 preparations were considered too low to be compatible with correct administration at room temperature. The 18% poloxamer 407 preparation displayed the thermogelling characteristics required.

The second objective of the study was to determine the adhesive properties of the short-chain fatty acid preparations. Previous studies have shown that poloxamer 407 formulations possess strong bioadhesive properties (23–26). In vitro techniques for determination of adhesion have been used to classify the adhesive strength of various polymers (27,28). The poor adhesion observed with the lowest poloxamer 407 concentration could be explained by the fluidity of the preparation at 37°C, whereas the adhesive properties of the gels containing 17.5% to 20% poloxamer 407 could be due to their viscosity. Indeed, it has been demonstrated that concentrated poloxamer 407 solutions, characterized by high viscosities, presented better adhesive properties to ileal mucosa (23) or an increased ocular contact time (22) than diluted preparations. The work of adhesion obtained with concentrated poloxamer 407 solutions was in the same range as those obtained with the best bioadhesive polymers like Carbopol 934P®, hydroxyethyl cellulose, and hydroxypropyl methyl cellulose (28). The three highest concentrations of poloxamer 407 could therefore provide adhesive short-chain fatty acid gels likely to prolong the residence time at the absorption site.

The third objective of the study was to determine to which extent poloxamer 407 gels could control the release of short-chain fatty acids. Due to its specific porosity (0.45 μm), the membrane used in the release assay acted as a sieve and was not a limiting factor for short-chain fatty acid diffusion. The release was about 2.5- and 5.5-fold higher for sodium propionate and acetate, respectively, than for sodium butyrate (data not shown). This result could be related to the size of short-chain fatty acid molecules, which have molecular weights (g/mol) as follows: sodium acetate 82, sodium propionate 96, and sodium butyrate 110. Due to their smaller size, sodium acetate and sodium propionate could diffuse more easily through the gels than the largest molecule, sodium butyrate. In addition, due to their higher solubility, sodium acetate and sodium propionate could diffuse more rapidly than sodium butyrate. Therefore, sodium butyrate was chosen as a tracer of diffusion. Compared to the rectal solution, the gel reduced significantly the sodium butyrate diffusion. At 60 min, the area under the curve was decreased by 60%. Therefore, gelling of the rectal solution allowed control of the short-chain fatty acid release. This effect could result in minimizing short-chain fatty acid loss in the colonic lumen and thereby improve bioavailability.

In conclusion, the formulation of short-chain fatty acid enemas could be optimized using an 18% poloxamer 407 solution as a vehicle. This concentration provided a preparation that was liquid at room temperature and accordingly was easily administrated rectally. The sol-gel transition temperature of 26.4°C could be compatible with the migration of the preparation toward the colon and its gelling in situ. At 37°C, the gel formed an adhesive system that could also control the short-chain fatty acid release rate.

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